

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
7 February 2002 (07.02.2002)

PCT

(10) International Publication Number
WO 02/10195 A2

(51) International Patent Classification: C07K 14/00

(21) International Application Number: PCT/CA01/01119

(22) International Filing Date: 2 August 2001 (02.08.2001)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data: 60/222,619 2 August 2000 (02.08.2000) US

(71) Applicant (for all designated States except US): THER-
ATECHNOLOGIES INC. [CA/CA]; 2310, Alfred-Nobel
Boulevard, Saint-Laurent, Québec H4S 2A4 (CA).

(72) Inventors; and

(75) Inventors/Applicants (for US only): GRAVEL, Denis
[CA/CA]; 207, des Pyrénées, St-Lambert, Québec J4S 1L3
(CA). HABI, Abdelkrim [CA/CA]; 7961, Champ d'Eau,
Anjou, Québec H1J 1X4 (CA). ABRIBAT, Thierry
[CA/CA]; 4659, Hutchison, Montréal, Québec H2V 4A2
(CA).

(74) Agent: SWABEY OGILVY RENAULT; Suite 1600,
1981 McGill College Avenue, Montréal, Québec H3A 2Y3
(CA).

(81) Designated States (national): AB, AG, AL, AM, AT, AU,
AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU,
CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH,
GM, HR, HU, ID, IL, IN, IS, JP, KB, KG, KP, KR, KZ, LC,
LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW,
MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK,
SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA,
ZW.

(84) Designated States (regional): ARIPO patent (GH, GM,
KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian
patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European
patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE,
IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF,
CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD,
TG).

Published:

— without international search report and to be republished
upon receipt of that report

For two-letter codes and other abbreviations, refer to the "Guid-
ance Notes on Codes and Abbreviations" appearing at the begin-
ning of each regular issue of the PCT Gazette.



WO 02/10195 A2

(54) Title: MODIFIED BIOLOGICAL PEPTIDES WITH INCREASED POTENCY

(57) Abstract: The present invention is concerned with modified biological peptides providing increased potency, prolonged activity and/or increased half-life thereof. The modification is made via coupling through an amide bond with at least one conformationally rigid substituent, either at the N-terminal of the peptide, the C-terminal of the peptide, on a free amino or carboxyl group along the peptide chain, or at a plurality of these sites. Those peptides exhibit clinical usefulness for example in treating states of insulin resistance associated with pathologies such as type II diabetes.

MODIFIED BIOLOGICAL PEPTIDES WITH INCREASED POTENCY

FIELD OF THE INVENTION

The present invention is concerned with modified peptides providing
5 increased biological potency, prolonged activity and/or increased half-life thereof.
The modification is made via coupling through an amide bond with at least one
conformationally rigid substituent either at the N-terminal of the peptide, the C-
terminal of the peptide, or a free amino or carboxyl group along the peptide chain, or
at a plurality of these sites.

BACKGROUND OF THE INVENTION

Most peptides are rapidly degraded in a serum medium and as a result,
their metabolites may sometimes end up with little or no residual biological activity.
To increase the activity of a peptide, various techniques have been proposed. One of
15 them is to anchor a hydrophobic chain at the N- or C-terminal of the peptidic
sequence or at other residues along the peptidic chain. This technique nevertheless
has limitations. For example, if the peptide comprises a long peptidic chain, the fact
that a small hydrophobic group is anchored to the N- or C-terminal does not
necessarily result in an increased activity of the peptide so-modified.

20 For example, it is known that substituting OH for a more hydrophobic
group like $-NEt_3$ at the C-terminal of a peptide sequence can result in a significantly
increased specific activity. However, these results are contradicted by several

publications, such as Muranichi et al. in *Pharm. Res.*, 1991, 8, 649-652, which stresses the inefficacy of a lauroyl group as a hydrophobic group at the N-terminal to increase activity. Accordingly, there does not seem to be any general rule or conclusion concerning biological potency, duration of activity and/or half life, that
5 can be derived as a result of the addition of substituents on a peptide chain, whether at the N- or C-terminal, or on certain residues along the peptidic chain.

US 6,020,311 discloses a hydrophobic growth hormone-releasing factor (GRF) analog wherein a rigidified hydrophobic moiety is coupled to the GRF peptide
10 via an amide bond at the N-terminal of the peptide. Such analog is said to have an improved anabolic potency with reduced dosage, and a prolonged activity. According to the teaching of this patent, however, the rigidified hydrophobic moiety always comprises a carbonyl group at one extremity, which means that an amide coupling thereof to the GRF can only take place at an amino site to form the required
15 amide bond. The patent does not mention, suggest or imply that similar results could be obtained if the amide coupling was made at the C-terminal by replacing the carbonyl group on the rigidified hydrophobic moiety with an amino group. The patent does not further mention, suggest or imply that the amide coupling could take place elsewhere on the peptide chain.

20

Biochemistry 2001, 40, pages 2860 to 2869 describes an hydrophobic glucagon-like peptide-1 (GLP-1) analog wherein hexenoic acid, a rigidified hydrophobic moiety is coupled to the GLP-1 peptide at the N-terminal of the peptide. The results show that

this analog exhibits a decreased affinity for the GLP-1 receptor, but an in vivo bioactivity similar to or slightly better than that of the wild type GLP-1, hypothetically because of increased resistance to serum degradation. According to this study, the linkage of acyl chains to His¹, amino-acid substitutions of Ala², and
5 the addition of amino-acid sequences at the N-terminal of the molecule would be better strategies to increase the in vivo biological activity than anchoring rigidified hydrophobic chains. However, most of these strategies involve a modification of the amino-acid composition of the natural molecule, which might have negative safety consequences for clinical applications, including the risks for immunogenicity and
10 side effects.

There is therefore a great need to develop peptides modified in a manner such that their activity will be increased, thereby improving their potency, i.e. greater resistance to serum degradation and/or from hyperagonistic properties, and/or
15 increasing their half-life without changing the amino-acid sequence that would be clinically safe and acceptable.

SUMMARY OF THE INVENTION

20 In accordance with the present invention, there is now provided a peptide of formula X_n-R₁ wherein:

- R₁ is a peptide sequence which cannot be the GRF sequence when X represents a trans-3-hexenoyl group attached at N-terminal position of the peptide sequence;

- each X' can be identical or independent from the others and is selected from the following list constituted by conformationally rigid moieties bearing:

- a) a carboxy or an amino group for coupling with the peptide sequence via an amide bond at the N-terminal of the peptide sequence, the C-terminal of the peptide sequence, at an available carboxy or amino site on the peptide sequence chain, and combinations thereof; and
- b) a carboxy group for coupling with the peptide sequence via an ester bond at an available hydroxy site on the peptide sequence chain, and combinations thereof;

wherein,

n is any digit between 1 to 5;

X being defined as:

- i) a straight, substituted C_1-C_{10} alkyl;
- ii) a branched, substituted C_1-C_{10} alkyl;
- iii) a straight or branched, unsubstituted or substituted C_1-C_{10} alkene;
- iv) a straight or branched, unsubstituted or substituted C_1-C_{10} alkyne;
- v) an unsubstituted or substituted, saturated or unsaturated C_3-C_{10} cycloalkyl or heterocycloalkyl wherein the heteroatom is O, S or N;
- vi) an unsubstituted or substituted C_5-C_{14} aryl or heteroaryl wherein the heteroatom is O, S or N;

wherein the substituent in the definitions i) to vi) comprises one or more

- a) straight or branched C_1-C_6 alkyl;
- b) straight or branched C_1-C_6 alkene;

b) straight or branched C_1-C_6 alkyne;

d) C_3-C_{10} cycloalkyl or heterocycloalkyl wherein at least 2 carbon atoms are optionally connected to the C_1-C_{10} alkyl, C_1-C_{10} alkene, C_1-C_{10} alkyne, C_3-C_{10} cycloalkyl or heterocycloalkyl, and C_5-C_{14} aryl or heteroaryl; or

5 e) C_5-C_{14} aryl or heteroaryl wherein at least 2 carbon atoms of the aryl or heteroaryl are optionally connected to the C_1-C_{10} alkyl, C_1-C_{10} alkene, C_1-C_{10} alkyne, C_3-C_{10} cycloalkyl or heterocycloalkyl, and C_5-C_{14} aryl or heteroaryl; and any isomers thereof, including cis and trans configurations, epimers, enantiomers, diastereoisomers, and racemic mixtures.

10

The term "aryl" includes phenyl, naphthyl and the like; the term "heterocycloalkyl" includes tetrahydrofuranyl, tetrahydrothiophanyl, tetrahydrothiopyranyl, tetrahydropyranyl and partially dehydrogenated derivatives thereof, azetidiny, piperidiny, pyrrolidiny, and the like; the term "heteroaryl" 15 comprises pyridiny, indoly, furanyl, imidazolyl, thiophanyl, pyrrolyl, quinoliny, isoquinoliny, pyrimidiny, oxazolyl, thiazolyl, isothiazolyl, isooxazolyl, pyrazolyl, and the like.

The expression "conformationally rigid moiety" means an entity having 20 limited conformational, i.e., rotational, mobility about its single bonds. Such mobility is limited, for example, by the presence of a double bond, a triple bond, or a saturated or unsaturated ring, which have little or no conformational mobility. As a result, the number of conformers or rotational isomers is reduced when compared, for

example, with the corresponding straight, unsubstituted and saturated aliphatic chain.

The conformationally rigid moiety may be hydrophobic, although this is not a prerequisite.

- 5 According to a preferred embodiment of the present invention the peptide sequence is selected from the group consisting of Growth hormone releasing factor (GRF), Somatostatin, Glucagon-like peptide 1 (7-37), amide human (GLP-1), hGLP-1 (7-36) NH₂, Parathyroid hormone fragments such as (PTH 1-34), Adrenocorticotrophic hormone (ACTH), Osteocalcin, Calcitonin, Corticotropin releasing factor, Dynorphin
- 10 A, β -Endorphin, Big Gastrin-1, GLP-2, Luteinizing hormone-releasing hormone, Melanocyte Stimulating Hormone (MSH), Atrial Natriuretic Peptide, Neuromedin B, Human Neuropeptide Y, Human Orexin A, Human Peptide YY, Human Secretin, Vasoactive Intestinal peptide (VIP), Antibiotic peptides (Magainin 1, Magainin 2, Cecropin A, and Cecropin B), Substance P (SP), Beta Casomorphin-5,
- 15 Endomorphin-2, Procolipase, Enterostatin, gastric inhibitory peptide, Chromogranin A, Vasostatin I & II, Procalcitonin, ProNCT, ProCGRP, IL8 (monocyte-derived), GCP-2, PF4, IP-10, MIG, SDF-1 α , GRO- α , I-TAC, RANTES, LD78, MIP-1 α , MCP-1, MCP-2, MCP-3, MCP-4, Eotaxin, MDC, and functional derivatives or fragments thereof.

20

DETAILED DESCRIPTION OF THE INVENTION

The amino acids are identified in the present application by the conventional three-letter abbreviations as indicated below, which are as generally

accepted, in the peptide art as recommended by the IUPAC-IUB commission in biochemical nomenclature:

	Alanine	Ala	Leucine	Leu
	Arginine	Arg	Lysine	Lys
5	Asparagine	Asn	Methionine	Met
	Aspartic acid	Asp	Phenylalanine	Phe
	Cysteine	Cys	Proline	Pro
	Glutamic acid	Glu	Serine	Ser
	Glutamine	Gln	Threonine	Thr
10	Glycine	Gly	Tryptophan	Trp
	Histidine	His	Tyrosine	Tyr
	Isoleucine	Ile	Valine	Val

All the peptide sequences set out herein are written according to the generally accepted convention whereby the N-terminal amino acid is on the left and the C-terminal amino acid is on the right.

The present invention relates to the use of at least one conformationally rigid moiety, to produce a new family of peptides with enhanced pharmacological properties.

The modified peptides of the present invention are prepared according to the following general method, well known in the art of solid phase synthesis.

Conformationally rigid moieties comprising a carboxy group are used for anchoring to amino groups such as those found on the lysine side chain as well

as the N-terminus of peptides. Those comprising an amino group are used for anchoring to carboxyl groups such as those found on the aspartic or glutamic acid side chains or the C-terminus of peptides. For such cases, the anchoring reaction is preferably performed on a solid phase support (Merrifield R.B. 1963, *J. Am. Chem. Soc.* 1963, 85, 2149 and *J. Am. Chem. Soc.*, 1964, 86, 304) using Benzotriazole-1-yl-oxy-tris (dimethylamino) phosphonium hexafluorophosphate described by Castro in the article (B. Castro et al., 1975, *Tetrahedron letters*, Vol. 14 :1219).

With respect to the anchoring dynamic, the preferred working temperatures are between 20°C and 60°C. The anchoring reaction time in the case of the more hydrophobic moieties, varies inversely with temperature, and varies between 0.1 and 24 hours.

Synthesis steps were carried out by solid-phase methodology on a manual peptide synthesizer using the Fmoc strategy. Fmoc amino acids were supplied by Chem Impex International Inc. Chicago and other commercial sources. Sequential Fmoc chemistry using BOP as coupling reagent was applied to the PL-Wang resin (Polymer Laboratories, catalog number : 1463-4799) for the production of the C-terminal carboxylic acid.

Fmoc deprotections were accomplished with piperidine 20% solution in DMF in three consecutive steps. Always under nitrogen scrubbing, a first solution of piperidine 20% was used for 1min. to remove the major part of the Fmoc

protecting groups. Then, the solution was drained, and another fresh piperidine 20% solution was introduced this time for 3min, drained again and finally another solution of piperidine 20% for 10min. The peptide-resin was then washed 4 times successively with 50 mL of DMF under nitrogen scrubbing. After completion of
5 the synthesis, the resin was well washed with DMF and DCM prior to drying.

Final cleavage of side chain protecting groups and peptide-resin bonds were performed using the following mixture: TFA, ethanedithiol, triisopropylsilane, thioanisole, phenol, water (92:1.66:1.66:1:1:2). A final
10 concentration of 20 mL of cleavage cocktail per gram of dried peptide-resin was used to cleave the peptide from the resin. The cleavage reaction was performed at room temperature for 2 hours. The free peptide, now in solution in the TFA cocktail, was then filtered on a coarse fritted disk funnel. The resin was then washed 3 times with pure TFA. The peptide/TFA mixture was evaporated under
15 vacuum on a Rotary evaporator, precipitated and washed with ether prior to its dissolution in water and freeze drying to eliminate the remaining traces of solvent and scavengers.

Coupling of the first Fmoc-amino acid to the Wang resin

20 We used 4-alkoxybenzyl alcohol polystyrene (Wang resin) and 2 eq of the desired Fmoc-amino acid in DMF and let both products mix together under nitrogen scrubbing for 15min at room temperature. Then 3.3 eq of pyridine and 2 eq of 2,6-dichlorobenzoylchloride were added successively and the reaction was

carried out under nitrogen scrubbing for 15-20 hours. (Seiber P., 1987, *Tetrahedron Letters*, Vol. 28, No. 49, pp 6147-6150). After this reaction, the reaction vessel was drained and the resin washed 4 times successively with DMF under nitrogen scrubbing. Any remaining hydroxyl groups of the resin were
5 benzoylated with 3 eq of benzoylchloride and pyridine in DCE (dichloroethane) for 2 hours.

Coupling of each remaining amino acid on the growing peptide

For each of the following Fmoc-amino acid we dissolved 3 eq of the
10 Fmoc-amino acid with 3 eq of BOP (Benzotriazole-1-yl-oxy-tris (dimethylamino) phosphonium hexafluorophosphate) (B. Castro et al., 1975, *Tetrahedron letters*, Vol. 14 :1219) in DMF, added the resulting solution to the resin in the reaction vessel, started the nitrogen scrubbing and added 6 eq of DIPEA (diisopropylethylamine) to start the coupling reaction. The coupling mixture was
15 scrubbed under nitrogen for 60 min. in the reaction vessel; then drained from the vessel, the resin was washed 3 times successively with DMF and a qualitative ninhydrin test was performed to verify completion of the reaction.

The coupling of the Fmoc-L-Lys(Aloc)-OH (PerSeptive Biosystems,
20 catalog number : GEN911209), Fmoc-L-Glu(OAl)-OH (PerSeptive Biosystems, catalog number : GEN911207) and Fmoc-L-Asp(OAl)-OH (PerSeptive Biosystems, catalog number : GEN911205) were carried out in the same way as for the Fmoc-amino acids as described above.

Deprotection of allylic groups

The peptide-resin (X mmol) was then introduced in DCM under nitrogen scrubbing and after 10 min. the $\text{PdCl}_2(\text{PPh}_3)_2$ (X mmol x 0.05 / 0.05 eq) (palladium(II) bis-triphenylphosphine) was added to the mixture (Bürger H., Kilian W., *J. Organometallics*, 1969, 18:299). Then the $(\text{CH}_3\text{CH}_2\text{CH}_2)_3\text{SnH}$ (X mmol x 6 / 6eq) (tributyltinhydride) was diluted in DCM and added dropwise to the peptide-resin suspension with an addition funnel over a period of 30 minutes. The reaction was continued for another 10 minutes then the vessel was drained from the cleavage mixture and right after the peptide-resin was washed 4 times with DCM and 4 times with DMF (Dangles O., Guibé F., Balavoine G., Lavielle S., Marquet A., 1987, *J. Org. Chem.*, 52: 4984).

Coupling of the conformationally rigid acids and alkylamines

The coupling of the conformationally rigid acids and amines to the side chains of the peptide-resin was conducted under the same conditions as those of the Fmoc-amino acids except that for these side chain modifications we used 10 equivalents of the rigid moieties and coupling reagent instead of 3.

The invention is not limited to any particular peptide sequence. Preferred peptide sequences R^1 comprise those with therapeutic properties, as well as functional derivatives or fragments thereof. The therapeutic properties of such peptides which may be used in accordance with the present invention include,

- without limitation, treatment of bone diseases including osteoporosis, postmenopausal osteoporosis and bone deposits, cancer treatment, regulating blood glucose, type II diabetes, treatment to to enhance mucosal regeneration in patients with intestinal diseases, treatment for diseases related to inflammatory responses,
- 5 obesity treatment, treatment for autism and pervasive development disorders, hyperproliferative skin conditions, aging, altering the proliferation of peripheral blood mononuclear cells, regulation of myometrial contractility and of prostaglandin release, stimulation of ACTH release, inhibition of interleukin-8 production, stimulation of acid release, enhancement of mucosal regeneration in
- 10 patients with intestinal diseases, treatment for hormone-dependent diseases and conditions including for hormone-dependent cancers, modulation of melanocyte information process, involved in pressure and volume homeostasis, regulation of exocrine and endocrine secretions, smooth muscle contraction, feeding, blood pressure, blood glucose, body temperature and cell growth, regulation of food
- 15 intake and energy balance, inhibition of cancer cell growth, stimulation of pancreatic secretion, or stimulate cell growth.

Growth hormone releasing factor (GRF):

- Xaa₁-Xaa₂-Asp-Ala-Ile-Phe-Thr-Xaa₄-Ser-Tyr-Arg-Lys-Xaa₁₃-Leu-Xaa₁₅-Gln-Leu-
- 20 Xaa₁₈-Ala-Arg-Lys-Leu-Leu-Xaa₂₄-Xaa₂₅-Ile-Xaa₂₇-Xaa₂₈-Arg-Gln-Gln-Gly-Glu-Ser-Asn-Gln-Glu-Arg-Gly-Ala-Arg-Ala-Arg-Leu-NH₂

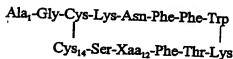
wherein,

Xaa₄ is Tyr or His;

Xaa₁₈ is Val or Ala;

- Xaa₁ is Asn or Ser;
 Xaa₁₁ is Val or Ile;
 Xaa₁₁ is Ala or Gly;
 Xaa₁₄ is Ser or Tyr;
 5 Xaa₁₄ is Gln or His;
 Xaa₁₇ is Asp or Glu;
 Xaa₁₇ is Met, Ile or Nle; and
 Xaa₂₁ is Ser or Asn.

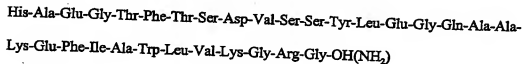
10 Somatostatin:



- 15 wherein,

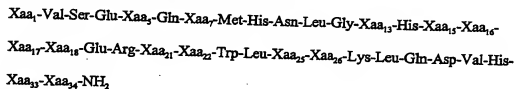
Xaa₁₂ is Tyr or Ser.

Glucagon-like peptide 1 (7-37), (amide human (hGLP-1)):



20

Parathyroid hormone fragments (PTH 1-34):



- 25 wherein,

Xaa₁ is Ser or Ala;

Xaa₃ is Ile or Met;

- Xaa₇ is Leu or Phe;
 Xaa₁₃ is Lys or Gln;
 Xaa₁₅ is Leu or Arg;
 Xaa₁₆ is Asn or Ala or Ser or His;
 5 Xaa₁₇ is Ser or Thr;
 Xaa₁₈ is Met or Val or Leu;
 Xaa₂₁ is Val or met or Gln;
 Xaa₂₂ is Gln or Gln or Asp;
 Xaa₂₃ is Arg or Gln;
 10 Xaa₂₄ is Lys or Met;
 Xaa₃₃ is Asn or Ser; and
 Xaa₃₄ is Phe or Ala.

Adrenocorticotrophic hormone (ACTH):

- 15 Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Xaa₁₃-Gly-Xaa₁₅-Lys-Arg-Arg-
 Pro-Xaa₂₀-Lys-Val-Tyr-Pro-Asn-Xaa₂₆-Xaa₂₇-Xaa₂₈-Xaa₂₉-Glu-Xaa₃₁-Xaa₃₂-Glu-
 Xaa₃₄-Xaa₃₅-Xaa₃₆-Xaa₃₇-Glu-Xaa₃₉-NH₂

wherein,

- Xaa₁₃ is Val or Met;
 20 Xaa₁₅ is Lys or Arg;
 Xaa₂₀ is Val or Ile;
 Xaa₂₆ is Gly or Ser;

Xaa₁₇ is Ala or Phe or Val;

Xaa₂₈ is Glu or Gln;

Xaa₂₉ is Asp or Asn or Glu;

Xaa₃₁ is Ser or Thr;

5 Xaa₃₃ is Ala or Val or Ser;

Xaa₃₄ is Ala or Asn or Gly;

Xaa₃₅ is Phe or Met;

Xaa₃₆ is Pro or Gly;

Xaa₃₇ is Leu or Val or Pro; and

10 Xaa₃₉ is Phe or Val or Leu.

Osteocalcin:

Tyr-Leu-Xaa₂₂-Xaa₂₃-Xaa₅₄-Leu-Gly-Ala-Pro-Xaa₅₉-Pro-Tyr-Pro-Asp-Pro-Leu-Glu-
Pro-Xaa₆₈-Arg-Glu-Val-Cys-Glu-Leu-Asn-Pro-Xaa₇₇-Cys-Asp-Glu-Leu-Ala-Asp-
His-Ile-Gly-Phe-Gln-Xaa₈₉-Ala-Tyr-Xaa₉₁-Arg-Xaa₉₄-Tyr-Gly-Xaa₉₇-Val-NH₂

15 wherein,

Xaa₂₂ is Tyr or Asp or Asn;

Xaa₅₃ is Gln or His or Asn;

Xaa₅₄ is Trp or Gly;

Xaa₅₉ is Val or Ala;

20 Xaa₆₈ is Arg or Lys or His;

Xaa₇₇ is Asp or Asn;

Xaa₈₉ is Glu or Asp;

Xaa₉₂ is Arg or Lys;

Xaa₉₄ is Phe or Ile; and

Xaa₉₇ is Pro or Thr.

5 **Calcitonin:**

Cys-Xaa₉₅-Xaa₉₇-Leu-Ser-Thr-Cys-Xaa₉₂-Leu-Gly-Xaa₉₅-Xaa₉₄-Xaa₉₇-Xaa₉₄-Xaa₉₉-
 Xaa₁₀₀-Xaa₁₀₁-Xaa₁₀₂-Xaa₁₀₃-Xaa₁₀₄-Thr-Xaa₁₀₆-Xaa₁₀₇-Xaa₁₀₈-Xaa₁₀₉-Xaa₁₁₀-Xaa₁₁₁-
 Gly-Xaa₁₁₃-Xaa₁₁₄-Xaa₁₁₅-Pro-NH₂

wherein,

10 Xaa₉₆ is Gly or Ser or Ala;

Xaa₉₇ is Asn or Ser;

Xaa₉₂ is Met or Val;

Xaa₉₅ is Thr or Lys;

Xaa₉₆ is Tyr or Leu;

15 Xaa₉₇ is Thr or Ser;

Xaa₉₈ is Gln or Lys;

Xaa₉₉ is Asp or Glu;

Xaa₁₀₀ is Phe or Leu;

Xaa₁₀₁ is Asn or His;

20 Xaa₁₀₂ is Lys or Asn;

Xaa₁₀₃ is Phe or Leu;

Xaa₁₀₄ is His or Gln;

Xaa₁₀₆ is Phe or Tyr;

Xaa₁₀₇ is Pro or Ser;

Xaa₁₀₈ is Gln or Gly or Arg;

Xaa₁₀₉ is Thr or Ile;

5 Xaa₁₁₀ is Ala or Gly or Ser or Asp or Asn;

Xaa₁₁₁ is Ile or Phe or Val or Thr;

Xaa₁₁₃ is Val or Ala or Ser;

Xaa₁₁₄ is Gly or Glu; and

Xaa₁₁₅ is Ala or Thr.

10

Corticotropin releasing factor:

Ser-Glu-Glu-Pro-Pro-Ile-Ser-Leu-Asp-Leu-thr-Phe-His-Leu-Leu-Arg-Glu-Val-Leu-
Glu-Met-Xaa₁₀₁-Xaa₁₀₂-Ala-Glu-Gln-Leu-Ala-Gln-Gln-Ala-His-Ser-Asn-Arg-Lys-
Leu-Met-Glu-Ile-Ile-NH₂

15 wherein,

Xaa₁₀₁ is Ala or Pro; and

Xaa₁₀₂ is Arg or Gly.

Dynorphin A:

H-Tyr-Gly-Gly-Phe-Leu-Arg-Arg-Ile-Arg-Pro-Lys-Leu-Lys-Trp-Asp-Asn-Gln-OH

20

β -Endorphin:

H-Tyr-Gly-Gly-Phe-Met-Thr-Xaa₂₄₅-Glu-Xaa₂₄₆-Ser-Gln-Thr-Pro-Leu-Xaa₂₅₁-Thr-
Leu-Phe-Lys-Asn-Ala-Ile-Xaa₂₅₅-Lys-Asn-Xaa₂₆₂-Xaa₂₆₃-Lys-Lys-Gly-Xaa₂₆₇-OH

wherein,

Xaa₂₄₃ is Ser or Pro;

Xaa₂₄₅ is Lys or Arg;

Xaa₂₅₁ is Val or Met;

5 Xaa₂₅₉ is Ile or Val;

Xaa₂₆₂ is Ala or Thr or Ser or Val;

Xaa₂₆₃ is Tyr or His; and

Xaa₂₆₇ is Glu or Leu or Gln or His.

10 **Big Gastrin-1:**

pXaa₂₉-Leu-Gly-Xaa₃₂-Gln-Xaa₃₄-Xaa₃₅-Xaa₃₆-Xaa₃₇-Xaa₃₈-Xaa₃₉-Ala-Asp-Xaa₄₂-
Xaa₄₃-Lys-Lys-Xaa₄₆-Xaa₄₇-Pro-Xaa₅₀-Xaa₅₀-Glu-Xaa₅₂-Glu-Glu-Xaa₅₅-Ala-Tyr-Gly-
Trp-Met-Asp-Phe-NH₂

wherein,

15 Xaa₂₉ is Glu or Gln;

Xaa₃₂ is Pro or Leu;

Xaa₃₄ is Gly or Asp;

Xaa₃₅ is Pro or Ser;

Xaa₃₆ is Pro or Gln;

20 Xaa₃₇ is His or Gln;

Xaa₃₈ is Leu or Met or Phe or Gln;

Xaa₃₉ is Val or Ile;

Xaa₇₂ is Pro or Leu;

Xaa₇₃ is Ser or Ala;

Xaa₇₆ is Gln or Glu;

Xaa₇₇ is Gly or Arg;

5 Xaa₇₉ is Trp or Pro or Arg;

Xaa₈₀ is Leu or Val or Met;

Xaa₈₂ is Glu or Lys; and

Xaa₈₃ is Glu or Ala.

10 GLP-2:

His-Ala-Asp-Gly-Ser-Phe-Xaa₁₃₂-Xaa₁₃₃-Xaa₁₃₄-Xaa₁₃₅-Xaa₁₃₆-Xaa₁₃₇-Xaa₁₃₈-Leu-Asp-
 Xaa₁₆₁-Xaa₁₆₂-Ala-Xaa₁₆₄-Xaa₁₆₅-Xaa₁₆₆-Phe-Xaa₁₆₈-Xaa₁₆₉-Trp-Xaa₁₇₁-Xaa₁₇₂-Xaa₁₇₃-
 Thr-Xaa₁₇₅-Xaa₁₇₆-Xaa₁₇₇-Xaa₁₇₈;

wherein,

15 Xaa₁₃₂ is Ser or Thr;

Xaa₁₃₃ is Asp or Ser;

Xaa₁₃₄ is Glu or Asp;

Xaa₁₃₅ is Met or Phe;

Xaa₁₃₆ is Asn or Ser;

20 Xaa₁₃₇ is Thr or Lys;

Xaa₁₃₈ is Ile or Val or Ala;

Xaa₁₆₁ is Asn or Ile or His or Ser;

- Xaa₁₆₃ is Leu or Lys;
Xaa₁₆₄ is Ala or Thr;
Xaa₁₆₅ is Arg or Gln or Lys;
Xaa₁₆₆ is Asp or Glu;
5 Xaa₁₆₈ is Ile or Leu;
Xaa₁₆₉ is Asn or Asp;
Xaa₁₇₁ is Leu or Ile;
Xaa₁₇₂ is Ile or Leu;
Xaa₁₇₃ is Gln or Asn or His;
10 Xaa₁₇₅ is Lys or Pro;
Xaa₁₇₆ is Ile or Val;
Xaa₁₇₇ is Thr or Lys; and
Xaa₁₇₈ is Asp or Glu.

15 **Luteinizing hormone-releasing hormone:**

Xaa₁-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-OH
wherein,

Xaa₁ is pGlu, 5-oxoPro or Gln.

20 **Melanocyte Stimulating Hormone (MSH):**

Ac-Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Val-NH₂

Atrial Natriuretic Peptide:

H-Ser-Leu-Arg-Arg-Ser-Ser-Cys-Phe-Gly-Gly-Arg-Xaa₁₃₃-Asp-Arg-Ile-Gly-Ala-
Gln-Ser-Xaa₁₄₂-Leu-Gly-Cys-Asn-Ser-Phe-Arg-Tyr-OH

wherein,

5 Xaa₁₃₃ is Met or Ile; and

 Xaa₁₄₂ is Gly or Ser.

Neuromedin B:

H-Gly-Asn-Leu-Trp-Ala-Thr-Gly-His-Phe-Met-NH₂

10

Human Neuropeptide Y:

H-Tyr-Pro-Ser-Lys-Pro-Asp-Asn-Pro-Gly-Glu-Asp-Ala-Pro-Ala-Glu-asp-Met-Ala-
Arg-Tyr-Tyr-Ser-Ala-Leu-Arg-His-Tyr-Ile-Asn-Leu-Ile-Thr-Arg-Gln-Arg-Tyr-NH₂

15 **Human Orexin A:**

pGlu-Pro-Leu-Pro-Asp-Cys-Cys-Arg-Gln-Lys-Thr-Cys-Ser-Cys-Arg-Leu-Tyr-Glu-
Leu-Leu-His-Gly-Ala-Gly-Asn-His-Ala-Ala-Gly-Ile-Leu-Thr-Leu-NH₂

Human Peptide YY:

20 H-Tyr-Pro-Ile-Lys-Pro-Glu-Ala-Pro-Gly-Glu-Asp-Ala-Ser-Pro-Glu-Glu-Leu-Asn-
Arg-Tyr-Tyr-Ala-Ser-Leu-Arg-His-Tyr-Leu-Asn-Leu-Val-Thr-Arg-Gln-Arg-Tyr-
NH₂

Human Secretin:

H-His-Ser-Asp-Gly-Thr-Phe-Thr-Ser-Glu-Leu-Ser-Arg-Leu-Arg-Glu-Gly-Ala-Arg-
Leu-Gln-Arg-Leu-Leu-Gln-Gly-Leu-Val-NH₂

5 Vasoactive Intestinal peptide (VIP):

H-His-Ser-Asp-Ala-Val-Phe-Thr-Asp-Asn-Tyr-Thr-Arg-Leu-Arg-Lys-Gln-Met-Ala-
Val-Lys-Lys-Tyr-Leu-Asn-Ser-Ile-Leu-Asn-NH₂

Antibiotic peptides such as:**10 Magainin 1:**

Gly-Ile-Gly-Lys-Phe-Leu-His-Ser-Ala-Gly-Lys-Phe-Gly-Lys-Ala-Phe-Val-
Gly-Glu-Ile-Met-Lys-Ser

Magainin 2:

Gly-Ile-Gly-Lys-Phe-Leu-His-Ser-Ala-Lys-Lys-Phe-Gly-Lys-Ala-Phe-Val-
15 Gly-Glu-Ile-Met-Asn-Ser

Cecropin A:

Lys-Trp-Lys-Val-Phe-Lys-Lys-Ile-Glu-Lys-Val-Gly-Gln-Ala-Thr-Gln-Ile-
Ala-Lys

Cecropin B:

Lys-Trp-Lys-Val-Phe-Lys-Lys-Ile-Glu-Lys-Met-Gly-Arg-Asn-Ile-Arg-Asn-
20 Gly-Ile-Val-Lys-Ala-Gly-Pro-Ala-Ile-Ala-Val-Leu-Gly-Glu-Ala-Lys-Ala-
Leu.

Substance P (SP):

Arg-Pro-Leu-Pro-Gln-Glu-Phe-Phe-Gly-Leu-Met-amide

Beta Casomorphin-5:

Tyr-Pro-Phe-Pro-Gly

5 **Endomorphin-2:**Tyr-Pro-Phe-Phe-NH₂**Procolipase:**

100 aa peptide (X1-Pro-X2-Pro-Arg....)

Enterostatin:

10 Val-Pro-Asp-Pro-Arg

Gastrin Inhibitory Peptide:

Tyr-Ala-Glu-Gly-Thr-Phe-Ile-Ser-Asp-Tyr-Ser-Ile-Ala- Met-Asp-Lys-Ile-His-
 Gln-Gln-Asp-Phe- Val-Asn-Trp-Leu- Leu-Ala-Gln-Lys-Gly-Lys-Lys-Asn-Asp-
 Trp-Lys-His-Asn- Ile-Thr-Gln

15 **Chromogranin A****Vasostatin I****Vasostatin II:**

Leu Pro Val Asn Ser Pro Met Asn Lys Gly Asp Thr Glu Val Met Lys Cys Ile
 Val Glu Val Ile Ser Asp Thr Leu Ser Lys Pro Ser Pro Met Pro Val Ser Gln Glu
 20 Cys Phe Glu Thr Leu Arg Gly Asp Glu Arg Ile Leu Ser Ile Leu Arg His Gln Asn
 Leu Leu Lys Glu Leu Gln Asp Leu Ala Leu Gln Gly Ala Lys Glu Arg Ala His
 Gln Gln Lys Lys His Ser Gly Phe Glu Asp Glu Leu Ser Glu Val Leu Glu Asn

Gln Ser Ser Gln Ala Glu Leu Lys Glu Ala Val Glu Glu Pro Ser Ser Lys Asp Val

Met Glu

Procalcitonin

ProNCT

5 **ProCGRP**

Chemokine family:

CXC-group:

10 **IL8(monocyte-derived):**

SerAlaLysGluLeuArgCysGlnCys...

GCP-2:

15 GlyProValSerAlaValLeuThrGluLeuArgCysThrCys...

PF4:

GluAlaGluGluAspGlyAspLeuGlnCysLeuCys...

20 **IP-10:**

ValProLeuSerArgThrValArgCCysThrCys...

25 **MIG:**

ThrProValValArgLysGlyArgCysSerCys...

SDF-1 α :

30 LysProValSerLeuSerTyrArgCysProCys...

GRO- α :

35 AlaProLeuAlaThrGluLeuArgCysGlnCys...

I-TAC:

PheProMetPheLysLysGlyArgCysLeuCys...

5 **CC-group:****RANTES:**10

SerProTyrSerSerAspThrThrProCys...

LD78:

AlaProLeuAlaAlaAspThrProThrAlaCys...

15 **MIP-1 α :**

AlaProMetGlySerAspProProThrAlaCys...

MCP-1:20

GlnProAspAlaIleAsnAlaProValThrCys...

MCP-2:25

GlnProSerAspValSerIleProIleThrCys...

MCP-3:30

GlnProValGlyIleTAsnSerThrThrCys...

MCP-4:

GlnProAspAlaLeuAspValProSerThrCys...

35 **Eotaxin:**

GlyProAlaSerValProThrThrCys...

MDC:40

GlyProTyrGlyAlaAsnMetGluAspSerValCys...
and functional derivatives or fragments thereof.

45

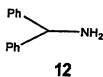
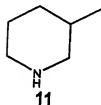
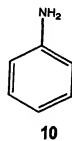
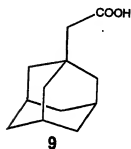
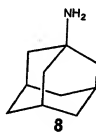
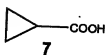
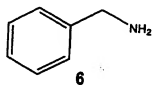
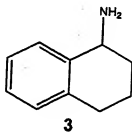
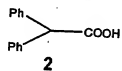
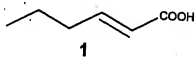
The complete definition of the previously listed sequences are known inter alia from
Mentlein, R (1999) Regul. Pept. 85:9-24 and from De Meester, I. Et al. (2000) Adv

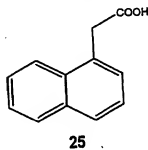
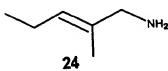
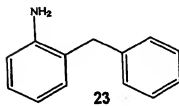
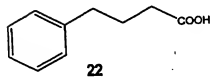
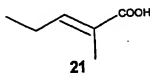
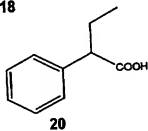
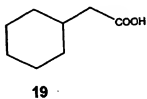
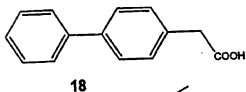
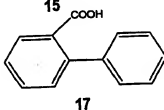
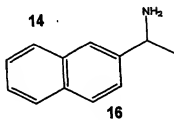
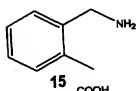
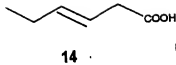
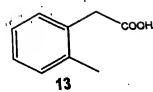
ExpMed Biol. 477:67-87. Those documents are incorporated by reference to the present application.

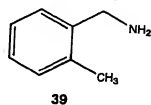
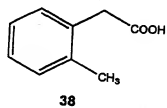
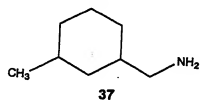
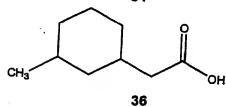
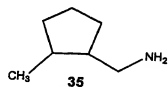
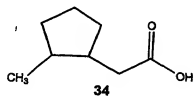
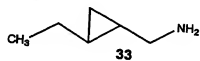
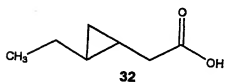
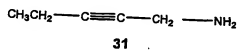
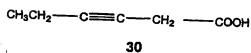
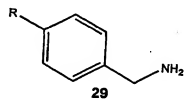
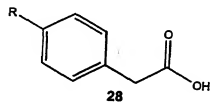
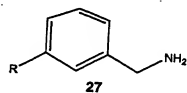
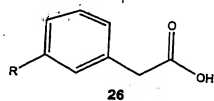
In a more preferred embodiment, the peptide is substituted with one or more
5 conformationally rigid moieties. Preferred structures of the conformationally rigid
moieties comprise those with a double bond, a triple bond or a saturated or
unsaturated ring.

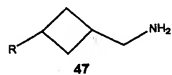
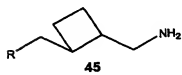
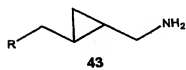
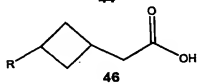
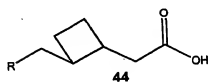
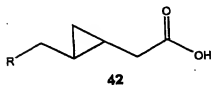
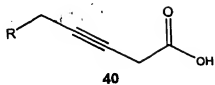
The following is a brief list of the formula of preferred conformationally
10 rigid moieties, identified as Formula 1 to 63, which are suitable for the purposes of
the present invention.

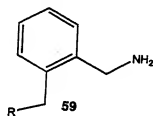
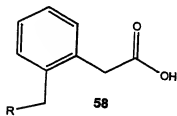
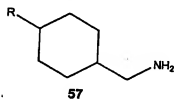
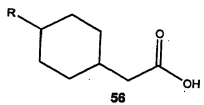
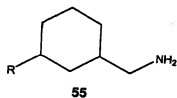
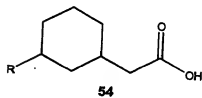
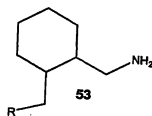
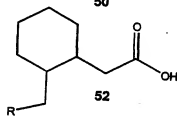
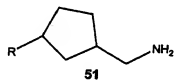
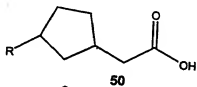
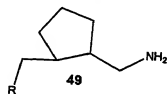
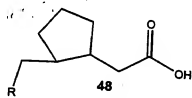
Among the preferred modified peptides according to the present invention,
are those wherein the peptide sequence is the sequence of a natural peptide.

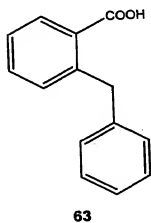
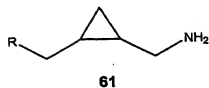
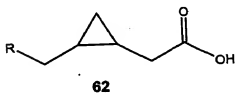
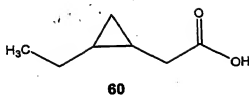










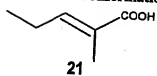
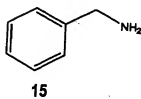
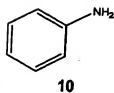
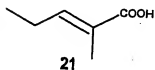


wherein, R is hydrogen, CH₃ or CH₂CH₃.

- 5 A preferred embodiment of the present invention is constituted by peptides wherein the peptide sequence is Somatostatin and at least one conformationally rigid moiety is coupled with said somatostatin peptide sequence via an amide bond at different positions as follows:

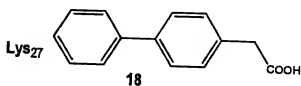
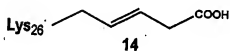
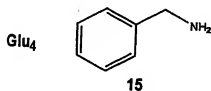
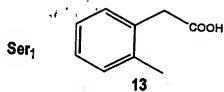
Position

conformationally rigid moieties

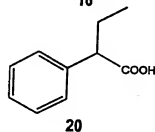
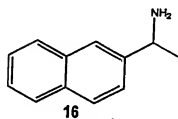
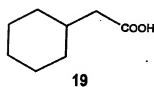
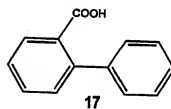
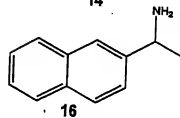
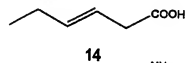
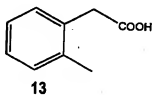
Ala₁Asp₅Cys₁₄Ala₁+Cys₁₄

Another preferred embodiment of the present invention is constituted by those peptides wherein the peptide sequence is PTH 1-34 and at least one conformationally rigid moiety is coupled with said PTH 1-34 peptide sequence via an amide bond at different positions as follows:

Position	conformationally rigid moieties
----------	---------------------------------



Ser₁+Lys₂₇



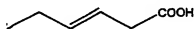
A further preferred embodiment of the present invention is constituted by those peptides wherein the peptide sequence is GLP-1 and at least one

conformationally rigid moiety is coupled with said GLP-1 peptide sequence via an amide bond at different positions as follows:

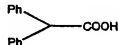
Position

conformationally rigid moieties

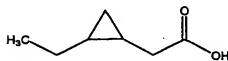
His₁



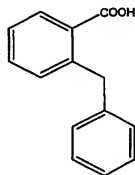
14



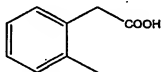
2



60

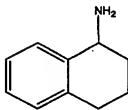


63

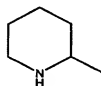


13

Glu₃



3

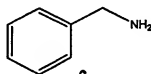


4

Asp₉



5

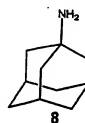


6

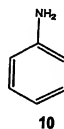
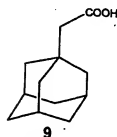
Position

conformationally rigid moieties

His₁ + Glu₃



His₁ + Asp₉



Glu₃ + Asp₉



Also preferred among the modified peptides according to the invention are those peptides wherein;

- 5 - the peptide sequence is GLP-2 and at least one conformationally rigid moiety is coupled with said GLP-2 peptide sequence via an amide or ester bond at different positions of the peptide sequence;
- the peptide sequence is Enterostatin and at least one conformationally rigid moiety is coupled with said Enterostatin peptide sequence via an amide bond at different positions of the peptide sequence;
- 10 - the peptide sequence is NPY and at least one conformationally rigid moiety is coupled with said NPY peptide sequence via an amide or ester bond at different positions of the peptide sequence;
- the peptide sequence is NPY Y and at least one conformationally rigid moiety is coupled with said NPY Y peptide sequence via an amide or ester bond at different positions of the peptide sequence;
- 15 - the peptide sequence is Secretin and at least one conformationally rigid moiety is coupled with said Secretin peptide sequence via an amide or ester bond at different positions of the peptide sequence;
- the peptide sequence is Vasoactive Intestinal Peptide and at least one conformationally rigid moiety is coupled with said Vasoactive Intestinal Peptide sequence via an amide or ester bond at different positions of the peptide sequence;
- 20

- the peptide sequence is Gastrin Inhibitory Peptide and at least one conformationally rigid moieties is coupled with said Gastrin Inhibitory Peptide sequence via an amide or ester bond at different positions of the peptide sequence;

5 - the peptide sequence is Vasostatin II and at least one conformationally rigid moiety is coupled with said Vasostatin II peptide sequence via an amide or ester bond at different positions of the peptide sequence;

- the peptide sequence is RANTES and at least one conformationally rigid moiety is coupled with said RANTES peptide sequence via an amide or ester bond at different positions of the peptide sequence;

10 - the peptide sequence is Eotaxin and at least one conformationally rigid moiety is coupled with said Eotaxin peptide sequence via an amide or ester bond at different positions of the peptide sequence.

15 In the modified peptides of the invention, the conformationally rigid moiety is preferably coupled with said peptide sequence via an amide bond at the N-terminal.

The modified peptides according to the invention, wherein the
20 conformationally rigid moiety is the formula referenced 60 in the description, are of a particular interest.

The modified peptides of the present invention can be administered in various ways, such as for example, intravenously, subcutaneously, intradermally,

transdermally, intraperitoneally, orally, or topically. The modified peptides of the present invention can also be administered by inhalation, when in a powder form or aerosol form. Furthermore, pharmaceutically acceptable carriers for delivery of modified peptides of the present invention include, without limitation, liposome,
5 nanosome, patch, implant or any delivery devices.

In addition to the carboxy and amino groups present at the C- and N-terminals respectively of the peptide, other carboxy and amino sites can be available on the peptide chain. For example, if the peptide chain comprises amino acids
10 provided with a carboxylic acid side chain such as aspartic acid and glutamic acid, additional carboxy sites will therefore be available on the chain for amidation. Should the peptide chain comprise amino acids with a carboxamide side chain such as asparagine and glutamine, these also provide additional carboxy groups for
15 amidation by a conformationally rigid moiety, provided that they are accessed synthetically via the corresponding aspartic and glutamic acids. Further, if the peptide comprises amino acids provided with a basic side chain such as arginine, histidine or lysine, additional amino sites will then be available on the chain for
20 amidation by a conformationally rigid moiety. The peptide chain may also include both acidic and basic amino acids, meaning that the conformationally rigid substituents could be coupled to the peptide chain via the N-terminal, the C-terminal,
a carboxy site on the peptide chain, an amino site on the peptide chain, or a plurality of these sites.

The present invention will be more readily understood by referring to the following examples which are given to illustrate the invention rather than to limit its scope.

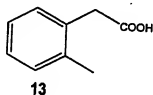
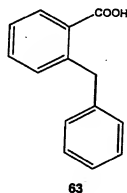
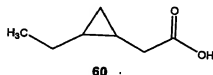
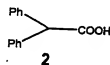
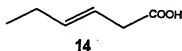
EXAMPLE 1

Synthesis of GLP-1 analogs

In accordance with the present invention, at least one of the following conformationally rigid moiety is coupled with the GLP-1 peptide sequence via an amide bond at different positions as follows.

Position conformationally rigid moieties

His₁



hGLP-1 (7-37) analogs synthesis

hGLP-1 (7-37) derivatives modified at the amino terminus with rigid hydrophobic moieties were synthesized using Fmoc chemistry (1), on the Symphony apparatus (Rainin Instrument Co., Inc.). Fmoc-Gly-Wang resin (0.70mmole/g) and five

equivalents of reagents (100µm scale, amino acids concentration of 200mM), were used with a time coupling of 30 minutes. The reactions have been monitored by the Kaiser test. The three conformationally rigid moieties introduced at the N-terminus of the hGLP-1 (7-37) are:

- 5 - **Peptide # 1** = (O-Tolylacetic acid-His³)-hGLP-1 (7-37) [O-Tolylacetic acid (13) (10 equivalents per coupling; coupling time 45 min)]
- **Peptide # 2** = ((+,-)-*cis*-2-Ethylcyclopropylacetic acid -His³)-hGLP-1 (7-37) [(+,-)-*cis*-2-Ethylcyclopropylacetic acid (60) (7.5 equivalents per coupling; coupling time 60 min)].

10

The peptides were cleaved using a TFA cocktail (92% TFA, 2% ethanedithiol, 2% thioanisole, 2% triisopropylsilane, 2% water, 2% (w/v) phenol) for 2 hours. All the analogs have been purified by reverse-phase HPLC. They have been analyzed by analytical HPLC and by MS (MALDI-TOF).

15

The synthesis of GLP-1 analogs is well known to the person skilled in the art and is further illustrated by the general references Fmoc Solid Phase Peptide Synthesis. A Practical Approach (2000). Chan, W.C. and White, P.D., Oxford University Press, New York, USA, 346p which are incorporated by reference.

20

Biological assess of GLP-1 analogs

Materials & Methods

25 **Oral Glucose Tolerance Test (OGTT)**

Six-week old female CD1 mice (Charles River) were fasted for at least 16 hours. Mice were given 1.5 mg of glucose per gram of body weight orally in water through a gastric gavage tube at $t = 0$ min and blood was collected from a tail vein at $t = 0, 10, 20, 30, 60, 90$ and 120 min for measurement of blood glucose using a glucose meter (Lifescan). Peptides or vehicle were injected subcutaneously 5 min prior to the glucose administration. Data were expressed as the area under the curve, calculated from the change (Δ) in blood glucose for each time, using the trapezoidal rule. Therefore, the data represent the integrated increase in blood glucose over a 120 min period following glucose administration. Data presented are the mean \pm SEM of 4 to 11 animals per group.

Test articles

All peptides, including wild-type GLP-1 (7-37), were tested in the OGTT test at 3 different concentrations: 1, 5 and 10 μ g per mouse. In a first set of experiments (study A), peptide 3 was tested in comparison with vehicle and hGLP-1 (7-37). In a second set of experiments (study B), peptides 1 and 2 were tested in comparison with vehicle and hGLP-1 (7-37).

wt GLP1: hGLP(7-37)

Peptide #1: (O-Tolylacetic acid-His³)-hGLP-1 (7-37)

Peptide #2: ((+,-)-cis-2-Ethylcyclopropylacetic acid-His³)-hGLP-1 (7-37)

Peptide #3: (Hexenoyl-trans-3-His³)-hGLP-1 (7-37)

Results and conclusions

Results are shown in Fig I(study A) and Fig.II (study B)

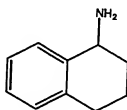
In studies A and B, administration of vehicle resulted in a similar integrated response in glucose levels (study A: 380 ± 57 vs study B: 309 ± 68 mMx120 min), illustrating the validity and reproducibility of the methodology. Although wt GLP-1 induced a dose-related decrease in the glucose response, this peptide was not able to completely suppress the glucose response at any dose, which might be

interpreted as a limitation in its potential clinical usefulness. In contrast, peptide 3 (study A, Fig.1) was able to completely abolish the glucose response, but only at the 10 ug dose (9 ± 26 mMx120 min). Surprisingly, peptide 2 (study B, Fig.2) was even more potent than peptide 3, being able to totally prevent the glucose response both at the 5 ug and the 10 ug doses (5 ug: -17 ± 67 mMx120 min; 10 ug: 61 ± 64 mMx120 min). In conclusion, the GLP-1 analog corresponding to peptide 2 was identified with marked increased biological potency over the wild type GLP-1 (7-37), because of this increased potency, this peptide may have clinical usefulness in treating states of insulin resistance associated with pathologies such as type II diabetes.

Position

conformationally rigid moieties

5

Glu₃

3

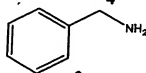


4

10

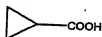
Asp₉

5

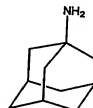


6

15

His₁ + Glu₃

7

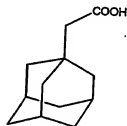


8

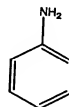
20

25

30

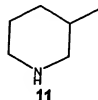
His₁ + Asp₉

9

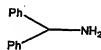


10

35

Glu₃ + Asp₉

11



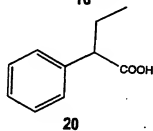
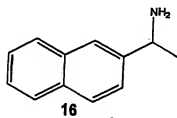
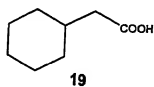
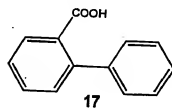
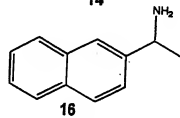
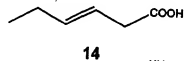
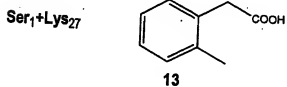
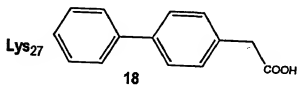
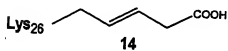
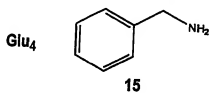
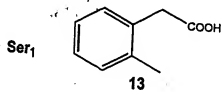
12

40

EXAMPLE 2**PTH 1-34 analogs**

In accordance with the present invention, at least one of the following
5 conformationally rigid moiety is coupled with the PTH 1-34 peptide sequence via an
amide bond at different positions as follows.

Position**conformationally rigid moieties**

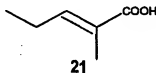


EXAMPLE 3**Somatostatin analogs**

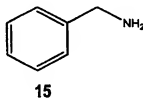
In accordance with the present invention, at least one of the following conformationally rigid moiety is coupled with the somatostatin peptide sequence via an amide bonds at different position as follows.

Position conformationally rigid moieties

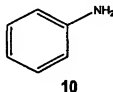
Ala₁



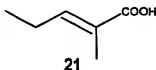
Asp₅



Cys₁₄



Ala₁+Cys₁₄



While the invention has been described in connection with specific
10 embodiments thereof, it will be understood that it is capable of further modifications,
and this application is intended to cover any variations, uses or adaptations of the
invention following, in general, the principles of the invention, and including such

departures from the present description as come within known or customary practice within the art to which the invention pertains, and as may be applied to the essential features hereinbefore set forth, and as follows in the scope of the appended claims.

WHAT IS CLAIMED IS:

1. A peptide of formula X_n-R_1 , wherein:

- R_1 is a peptide sequence, a functional analog thereof or a fragment thereof;
each X can be identical or independent from the others and is selected from the following list constituted by conformationally rigid moieties:
 - i) a straight, substituted C_1-C_{10} alkyl;
 - ii) a branched, substituted C_1-C_{10} alkyl;
 - iii) a straight or branched, unsubstituted or substituted C_1-C_{10} alkene;
 - iv) a straight or branched, unsubstituted or substituted C_1-C_{10} alkyne;
 - v) an unsubstituted or substituted, saturated or unsaturated C_3-C_{10} cycloalkyl or heterocycloalkyl wherein the heteroatom is O, S or N;
 - vi) an unsubstituted or substituted C_5-C_{14} aryl or heteroaryl wherein the heteroatom is O, S or N;

wherein the substituent in the definitions i) to vi) comprises one or more

- a) straight or branched C_1-C_6 alkyl;
- b) straight or branched C_1-C_6 alkene;
- c) straight or branched C_1-C_6 alkyne;
- d) C_3-C_{10} cycloalkyl or heterocycloalkyl wherein at least 2 carbon atoms are optionally connected to the C_1-C_{10} alkyl, C_1-C_{10} alkene, C_1-C_{10} alkyne, C_3-C_{10} cycloalkyl or heterocycloalkyl, and C_5-C_{14} aryl or heteroaryl; or
- e) C_5-C_{14} aryl or heteroaryl wherein at least 2 carbon atoms of the aryl or heteroaryl are optionally connected to the C_1-C_{10} alkyl, C_1-C_{10} alkene, C_1-C_{10}

alkyl, C_3 - C_{10} cycloalkyl or heterocycloalkyl, and C_3 - C_{14} aryl or heteroaryl, said group X also comprising at least one group selected from:

- α) a carboxy or an amino group for coupling with the peptide sequence via an amide bond at the N-terminal of the peptide sequence, the C-terminal of the peptide sequence, at an available carboxy or amino site on the peptide sequence chain, and combinations thereof; and
- β) a carboxy group for coupling with the peptide sequence via an ester bond at an available hydroxy site on the peptide sequence chain, and combinations thereof;

wherein,

n is any digit between 1 to 5;

and any isomers thereof, including cis and trans configurations, epimers, enantiomers, diastereoisomers, and racemic mixtures,

the peptides defined in claim 1 of U.S. Patent No. 6,020,311 being excluded.

2. A peptide as claimed in claim 1 wherein the peptide sequence is selected from the group consisting of Growth hormone releasing factor (GRF), Somatostatin, Glucagon-like peptide 1 (7-37), amide human (GLP-1) hGLP-1 (7-36) NH_2 , Parathyroid hormone fragments (PTH 1-34), Adrenocorticotrophic hormone (ACTH), Osteocalcin, Calcitonin, Corticotropin releasing factor, Dynorphin A, β -Endorphin, Big Gastrin-1, GLP-2, Luteinizing hormone-releasing hormone, Melanocyte Stimulating Hormone (MSH), Atrial Natriuretic Peptide, Neuromedin B, Human

Neuropeptide Y, Human Orexin A, Human Peptide YY, Human Secretin, Vasoactive Intestinal peptide (VIP), Antibiotic peptides (Magainin 1, Magainin 2, Cecropin A, and Cecropin B), Substance P (SP), Beta Casomorphin-5, Endomorphin-2, Procolipase, Enterostatin, gastric inhibitory peptide, Chromogranin A, Vasostatin I & II, Procalcitonin, ProNCT, CGRP (Calcitonin Gene Related Peptide), IL8 (monocyte-derived), GCP-2, PF4, IP-10, MIG, SDF-1 α , GRO- α , I-TAC, RANTES, LD78, MIP-1 α , MCP-1, MCP-2, MCP-3, MCP-4, Eotaxin, MDC, and functional analogs and derivatives or fragments thereof.

3. A peptide as claimed in claim 1 or 2 wherein the conformationally rigid moiety comprises at least a double bond, a triple bond or a saturated or unsaturated ring.

4. A peptide as claimed in any one of claims 1 to 3 wherein the conformationally rigid moiety comprises one or more of the structures of Formula 1 to 63 as defined in the description.

5. A peptide as claimed in any one of claims 1 to 4 wherein the peptide sequence is selected from the group consisting of:

Growth hormone releasing factor (GRF):

Xaa₁-Xaa₂-Asp-Ala-Ile-Phe-Thr-Xaa₄-Ser-Tyr-Arg-Lys-Xaa₁₃-Leu-Xaa₁₅-Gln-Leu-Xaa₁₈-Ala-Arg-Lys-Leu-Leu-Xaa₂₄-Xaa₂₅-Ile-Xaa₂₇-Xaa₂₈-Arg-Gln-Gln-Gly-Glu-Ser-Asn-Gln-Glu-Arg-Gly-Ala-Arg-Ala-Arg-Leu-NH₂

wherein,

Xaa_i is Tyr or His;

Ala₁-Gly-Cys-Lys-Asn-Phe-Phe-Trp
Cys₁₄-Ser-Xaa₁₂-Phe-Thr-Lys

Xaa₁₂ is Tyr or Ser;

His-Ala-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln-Ala-Ala-Lys-Glu-Phe-Ile-Ala-Trp-Leu-Val-Lys-Gly-Arg-Gly-OH(NH₂)

Xaa₁-Val-Ser-Glu-Xaa₂-Gln-Xaa₃-Met-His-Asn-Leu-Gly-Xaa₄-His-Xaa₅-Xaa₆-
Xaa₁₇-Xaa₁₈-Glu-Arg-Xaa₂₁-Xaa₂₂-Trp-Leu-Xaa₂₃-Xaa₂₄-Lys-Leu-Gln-Asp-Val-His-
Xaa₃₃-Xaa₃₄-NH₂

wherein,

Xaa₁ is Ser or Ala;
 Xaa₂ is Ile or Met;
 Xaa₇ is Leu or Phe;
 Xaa₁₃ is Lys or Glu;
 Xaa₁₉ is Leu or Arg;
 Xaa₁₆ is Asn or Ala or Ser or His;
 Xaa₁₇ is Ser or Thr;
 Xaa₁₈ is Met or Val or Leu;
 Xaa₂₁ is Val or met or Gln;
 Xaa₂₂ is Glu or Gln or Asp;
 Xaa₂₅ is Arg or Gln;
 Xaa₂₆ is Lys or Met;
 Xaa₃₃ is Asn or Ser; and
 Xaa₃₄ is Phe or Ala;

Adrenocorticotrophic hormone (ACTH):

Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Xaa₁₃-Gly-Xaa₁₅-Lys-Arg-Arg-
 Pro-Xaa₂₀-Lys-Val-Tyr-Pro-Asn-Xaa₂₆-Xaa₂₇-Xaa₂₈-Xaa₂₉-Glu-Xaa₃₁-Xaa₃₂-Glu-
 Xaa₃₄-Xaa₃₅-Xaa₃₆-Xaa₃₇-Glu-Xaa₃₉-NH₂

wherein,

Xaa₁₃ is Val or Met;
 Xaa₁₅ is Lys or Arg;

Xaa₂₀ is Val or Ile;
Xaa₂₆ is Gly or Ser;
Xaa₂₇ is Ala or Phe or Val;
Xaa₂₈ is Glu or Gln;
Xaa₂₉ is Asp or Asn or Glu;
Xaa₃₁ is Ser or Thr;
Xaa₃₂ is Ala or Val or Ser;
Xaa₃₄ is Ala or Asn or Gly;
Xaa₃₅ is Phe or Met;
Xaa₃₆ is Pro or Gly;
Xaa₃₇ is Leu or Val or Pro; and
Xaa₃₉ is Phe or Val or Leu;

Osteocalcin:

Tyr-Leu-Xaa₂₂-Xaa₂₃-Xaa₂₄-Leu-Gly-Ala-Pro-Xaa₂₅-Pro-Tyr-Pro-Asp-Pro-Leu-Glu-
Pro-Xaa₂₆-Arg-Glu-Val-Cys-Glu-Leu-Asn-Pro-Xaa₂₇-Cys-Asp-Glu-Leu-Ala-Asp-
His-Ile-Gly-Phe-Gln-Xaa₂₈-Ala-Tyr-Xaa₂₉-Arg-Xaa₃₀-Tyr-Gly-Xaa₃₁-Val-NH₂
wherein,

Xaa₂₂ is Tyr or Asp or Asn;
Xaa₂₃ is Gln or His or Asn;
Xaa₂₄ is Trp or Gly;
Xaa₂₉ is Val or Ala;

Xaa₆₈ is Arg or Lys or His;

Xaa₇₇ is Asp or Asn;

Xaa₈₉ is Glu or Asp;

Xaa₉₂ is Arg or Lys;

Xaa₉₄ is Phe or Ile; and

Xaa₉₇ is Pro or Thr;

Calcitonin:

Cys-Xaa₄₆-Xaa₄₇-Leu-Ser-Thr-Cys-Xaa₅₂-Leu-Gly-Xaa₅₇-Xaa₅₈-Xaa₅₉-Xaa₆₀-Xaa₆₁-Xaa₆₂-Xaa₆₃-Xaa₆₄-Xaa₆₅-Xaa₆₆-Xaa₆₇-Xaa₆₈-Xaa₆₉-Xaa₇₀-Xaa₇₁-Xaa₇₂-Xaa₇₃-Xaa₇₄-Xaa₇₅-Xaa₇₆-Xaa₇₇-Xaa₇₈-Xaa₇₉-Xaa₈₀-Xaa₈₁-Xaa₈₂-Xaa₈₃-Xaa₈₄-Xaa₈₅-Xaa₈₆-Xaa₈₇-Xaa₈₈-Xaa₈₉-Xaa₉₀-Xaa₉₁-Xaa₉₂-Xaa₉₃-Xaa₉₄-Xaa₉₅-Xaa₉₆-Xaa₉₇-Xaa₉₈-Xaa₉₉-Xaa₁₀₀-Xaa₁₀₁-Xaa₁₀₂-Xaa₁₀₃-Xaa₁₀₄-Thr-Xaa₁₀₆-Xaa₁₀₇-Xaa₁₀₈-Xaa₁₀₉-Xaa₁₁₀-Xaa₁₁₁-Gly-Xaa₁₁₃-Xaa₁₁₄-Xaa₁₁₅-Pro-NH₂

wherein,

Xaa₄₆ is Gly or Ser or Ala;

Xaa₈₇ is Asn or Ser;

Xaa₉₂ is Met or Val;

Xaa₉₅ is Thr or Lys;

Xaa₉₆ is Tyr or Leu;

Xaa₉₇ is Thr or Ser;

Xaa₉₈ is Gln or Lys;

Xaa₉₉ is Asp or Glu;

Xaa₁₀₀ is Phe or Leu;

Xaa₁₀₁ is Asn or His;

Xaa₁₀₂ is Lys or Asn;

Xaa₁₀₃ is Phe or Leu;

Xaa₁₀₄ is His or Gln;

Xaa₁₀₆ is Phe or Tyr;

Xaa₁₀₇ is Pro or Ser;

Xaa₁₀₈ is Gln or Gly or Arg;

Xaa₁₀₉ is Thr or Ile;

Xaa₁₁₀ is Ala or Gly or Ser or Asp or Asn;

Xaa₁₁₁ is Ile or Phe or Val or Thr;

Xaa₁₁₃ is Val or Ala or Ser;

Xaa₁₁₄ is Gly or Glu; and

Xaa₁₁₅ is Ala or Thr;

Corticotropin releasing factor:

Ser-Glu-Glu-Pro-Pro-Ile-Ser-Leu-Asp-Leu-thr-Phe-His-Leu-Leu-Arg-Glu-Val-Leu-
Glu-Met-Xaa₁₀₁-Xaa₁₀₂-Ala-Glu-Gln-Leu-Ala-Gln-Gln-Ala-His-Ser-Asn-Arg-Lys-
Leu-Met-Glu-Ile-Ile-NH₂

wherein,

Xaa₁₀₁ is Ala or Pro; and

Xaa₁₀₂ is Arg or Gly;

Dynorphin A:

H-Tyr-Gly-Gly-Phe-Leu-Arg-Arg-Ile-Arg-Pro-Lys-Leu-Lys-Trp-Asp-Asn-Gln-OH

β -Endorphin:

H-Tyr-Gly-Gly-Phe-Met-Thr-Xaa₂₄₃-Glu-Xaa₂₄₅-Ser-Gln-Thr-Pro-Leu-Xaa₂₅₁-Thr-
Leu-Phe-Lys-Asn-Ala-Ile-Xaa₂₅₉-Lys-Asn-Xaa₂₆₂-Xaa₂₆₃-Lys-Lys-Gly-Xaa₂₆₇-OH

wherein,

Xaa₂₄₃ is Ser or Pro;

Xaa₂₄₅ is Lys or Arg;

Xaa₂₅₁ is Val or Met;

Xaa₂₅₉ is Ile or Val;

Xaa₂₆₂ is Ala or Thr or Ser or Val;

Xaa₂₆₃ is Tyr or His; and

Xaa₂₆₇ is Glu or Leu or Gln or His;

Big Gastrin-1:

pXaa₅₉-Leu-Gly-Xaa₆₂-Gln-Xaa₆₄-Xaa₆₅-Xaa₆₆-Xaa₆₇-Xaa₆₈-Xaa₆₉-Ala-Asp-Xaa₇₂-
Xaa₇₃-Lys-Lys-Xaa₇₆-Xaa₇₇-Pro-Xaa₇₉-Xaa₈₀-Glu-Xaa₈₂-Glu-Glu-Xaa₈₃-Ala-Tyr-Gly-
Trp-Met-Asp-Phe-NH₂

wherein,

Xaa₅₉ is Glu or Gln;

Xaa₆₂ is Pro or Leu;

Xaa₆₄ is Gly or Asp;

Xaa₆₅ is Pro or Ser;

Xaa₆₆ is Pro or Gln;

Xaa₆₇ is His or Gln;

Xaa₆₈ is Leu or Met or Phe or Gln;

Xaa₆₉ is Val or Ile;

Xaa₇₂ is Pro or Leu;

Xaa₇₃ is Ser or Ala;

Xaa₇₆ is Gln or Glu;

Xaa₇₇ is Gly or Arg;

Xaa₇₉ is Trp or Pro or Arg;

Xaa₈₀ is Leu or Val or Met;

Xaa₈₂ is Glu or Lys; and

Xaa₈₃ is Glu or Ala;

GLP-2:

His-Ala-Asp-Gly-Ser-Phe-Xaa₁₃₂-Xaa₁₃₃-Xaa₁₃₄-Xaa₁₃₅-Xaa₁₃₆-Xaa₁₃₇-Xaa₁₃₈-Leu-Asp-
Xaa₁₆₁-Xaa₁₆₂-Ala-Xaa₁₆₄-Xaa₁₆₅-Xaa₁₆₆-Phe-Xaa₁₆₈-Xaa₁₆₉-Trp-Xaa₁₇₁-Xaa₁₇₂-Xaa₁₇₃-
Thr-Xaa₁₇₅-Xaa₁₇₆-Xaa₁₇₇-Xaa₁₇₈;

wherein,

Xaa₁₃₂ is Ser or Thr;

Xaa₁₃₃ is Asp or Ser;

Xaa₁₃₄ is Glu or Asp;

Xaa₁₃₅ is Met or Phe;

Xaa₁₅₆ is Asn or Ser;
Xaa₁₅₇ is Thr or Lys;
Xaa₁₅₈ is Ile or Val or Ala;
Xaa₁₆₁ is Asn or Ile or His or Ser;
Xaa₁₆₂ is Leu or Lys;
Xaa₁₆₄ is Ala or Thr;
Xaa₁₆₅ is Arg or Gln or Lys;
Xaa₁₆₆ is Asp or Glu;
Xaa₁₆₈ is Ile or Leu;
Xaa₁₆₉ is Asn or Asp;
Xaa₁₇₁ is Leu or Ile;
Xaa₁₇₂ is Ile or Leu;
Xaa₁₇₃ is Gln or Asn or His;
Xaa₁₇₅ is Lys or Pro;
Xaa₁₇₆ is Ile or Val;
Xaa₁₇₇ is Thr or Lys; and
Xaa₁₇₈ is Asp or Glu;

Luteinizing hormone-releasing hormone:

Xaa₁-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-OH

wherein,

Xaa₁ is pGlu, 5-oxoPro or Gln.

Melanocyte Stimulating Hormone (MSH):

Ac-Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Val-NH₂

Atrial Natriuretic Peptide:

H-Ser-Leu-Arg-Arg-Ser-Ser-Cys-Phe-Gly-Gly-Arg-Xaa₁₃₃-Asp-Arg-Ile-Gly-Ala-
Gln-Ser-Xaa₁₄₂-Leu-Gly-Cys-Asn-Ser-Phe-Arg-Tyr-OH

wherein,

Xaa₁₃₃ is Met or Ile; and

Xaa₁₄₂ is Gly or Ser;

Neuromedin B:

H-Gly-Asn-Leu-Trp-Ala-Thr-Gly-His-Phe-Met-NH₂

Human Neuropeptide Y:

H-Tyr-Pro-Ser-Lys-Pro-Asp-Asn-Pro-Gly-Glu-Asp-Ala-Pro-Ala-Glu-asp-Met-Ala-
Arg-Tyr-Tyr-Ser-Ala-Leu-Arg-His-Tyr-Ile-Asn-Leu-Ile-Thr-Arg-Gln-Arg-Tyr-NH₂

Human Orexin A:

pGlu-Pro-Leu-Pro-Asp-Cys-Cys-Arg-Gln-Lys-Thr-Cys-Ser-Cys-Arg-Leu-Tyr-Glu-
Leu-Leu-His-Gly-Ala-Gly-Asn-His-Ala-Ala-Gly-Ile-Leu-Thr-Leu-NH₂

Human Peptide YY:

H-Tyr-Pro-Ile-Lys-Pro-Glu-Ala-Pro-Gly-Glu-Asp-Ala-Ser-Pro-Glu-Glu-Leu-Asn-Arg-Tyr-Tyr-Ala-Ser-Leu-Arg-His-Tyr-Leu-Asn-Leu-Val-Thr-Arg-Gln-Arg-Tyr-NH₂

Human Secretin:

H-His-Ser-Asp-Gly-Thr-Phe-Thr-Ser-Glu-Leu-Ser-Arg-Leu-Arg-Gly-Ala-Arg-Leu-Gln-Arg-Leu-Leu-Gln-Gly-Leu-Val-NH₂

Vasoactive Intestinal peptide (VIP):

H-His-Ser-Asp-Ala-Val-Phe-Thr-Asp-Asn-Tyr-Thr-Arg-Leu-Arg-Lys-Gln-Met-Ala-Val-Lys-Lys-Tyr-Leu-Asn-Ser-Ile-Leu-Asn-NH₂

Antibiotic peptides such as:**Magainin 1:**

Gly-Ile-Gly-Lys-Phe-Leu-His-Ser-Ala-Gly-Lys-Phe-Gly-Lys-Ala-Phe-Val-Gly-Glu-Ile-Met-Lys-Ser

Magainin 2:

Gly-Ile-Gly-Lys-Phe-Leu-His-Ser-Ala-Lys-Lys-Phe-Gly-Lys-Ala-Phe-Val-Gly-Glu-Ile-Met-Asn-Ser

Cecropin A:

Lys-Trp-Lys-Val-Phe-Lys-Lys-Ile-Glu-Lys-Val-Gly-Gln-Ala-Thr-Gln-Ile-Ala-Lys

Cecropin B:

Lys-Trp-Lys-Val-Phe-Lys-Lys-Ile-Glu-Lys-Met-Gly-Arg-Asn-Ile-Arg-Asn-Gly-
Ile-Val-Lys-Ala-Gly-Pro-Ala-Ile-Ala-Val-Leu-Gly-Glu-Ala-Lys-Ala-Leu .

Substance P (SP):

Arg-Pro-Leu-Pro-Gin-Glu-Phe-Phe-Gly-Leu-Met-amide

Beta Casomorphin-5:

Tyr-Pro-Phe-Pro-Gly

Endomorphin-2:

Tyr-Pro-Phe-Phe-NH₂

Procolipase:

100 aa peptide (X1-Pro-X2-Pro-Arg,...)

Enterostatin:

Val-Pro-Asp-Pro-Arg

Gastrin Inhibitory Peptide:

Tyr-Ala-Glu-Gly-Thr-Phe-Ile-Ser-Asp-Tyr-Ser-Ile-Ala- Met-Asp-Lys-Ile-His-Gln-
Gln-Asp-Phe- Val-Asn-Trp-Leu- Leu-Ala-Gln-Lys-Gly-Lys-Lys-Asn-Asp-Trp-Lys-
His-Asn- Ile-Thr-Gln

Chromogranin A

Vasostatin I

Vasostatin II:

Leu Pro Val Asn Ser Pro Met Asn Lys Gly Asp Thr Glu Val Met Lys Cys Ile Val
Glu Val Ile Ser Asp Thr Leu Ser Lys Pro Ser Pro Met Pro Val Ser Gln Glu Cys Phe
Glu Thr Leu Arg Gly Asp Glu Arg Ile Leu Ser Ile Leu Arg His Gln Asn Leu Leu

Lys Glu Leu Gln Asp Leu Ala Leu Gln Gly Ala Lys Glu Arg Ala His Gln Gln Lys
Lys His Ser Gly Phe Glu Asp Glu Leu Ser Glu Val Leu Glu Asn Gln Ser Ser Gln
Ala Glu Leu Lys Glu Ala Val Glu Glu Pro Ser Ser Lys Asp Val Met Glu

Procalcitonin

ProNCT

ProCGRP

Chemokine family:

CXC-group:

IL8(monocyte-derived):

SerAlaLysGluLeuArgCysGlnCys...

GCP-2:

GlyProValSerAlaValLeuThrGluLeuArgCysThrCys...

PF4:

GluAlaGluGluAspGlyAspLeuGlnCysLeuCys...

IP-10:

ValProLeuSerArgThrValArgCCysThrCys...

MIG:

ThrProValValArgLysGlyArgCysSerCys...

SDF-1 α :

LysProValSerLeuSerTyrArgCysProCys...

GRO- α :

AlaProLeuAlaThrGluLeuArgCysGlnCys...

I-TAC:

PheProMetPheLysLysGlyArgCysLeuCys...

CC-group:

RANTES:

SerProTyrSerSerAspThrThrProCys...

LD78:

AlaProLeuAlaAlaAspThrProThrAlaCys...

MIP-1 α :

AlaProMetGlySerAspProProThrAlaCys...

MCP-1:

GlnProAspAlaIleAsnAlaProValThrCys...

MCP-2:

GlnProSerAspValSerIleProIleThrCys...

MCP-3:

GlnProValGlyIleTAsnSerThrThrCys...

MCP-4:

GlnProAspAlaLeuAspValProSerThrCys...

Eotaxin:

GlyProAlaSerValProThrThrCys...

MDC:

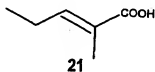
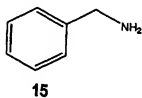
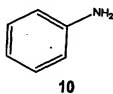
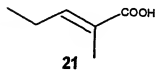
GlyProTyrGlyAlaAsnMetGluAspSerValCys...

and functional analogs and derivatives or fragments thereof.

6. A peptide according to claim 5 wherein the peptide sequence is the sequence of a natural peptide and functional analog or a fragment thereof or a clinically safe and acceptable derivative or analog thereof.

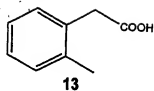
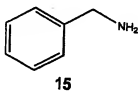
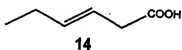
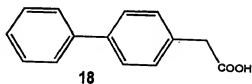
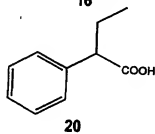
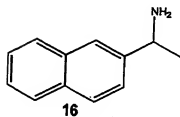
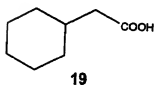
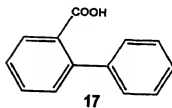
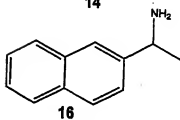
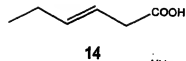
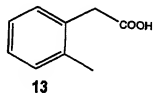
7. A peptide as claimed in claim 1 wherein the peptide sequence is Somatostatin and at least one conformationally rigid moiety is coupled with said somatostatin peptide sequence via an amide bond at different positions as follows:

Position, conformationally rigid moieties

Ala₁Asp₅Cys₁₄Ala₁+Cys₁₄

8. A peptide as claimed in claim 1 wherein the peptide sequence is PTH 1-34 and at least one conformationally rigid moiety is coupled with said PTH 1-34 peptide sequence via an amide bond at different positions as follows:

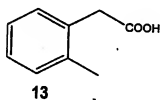
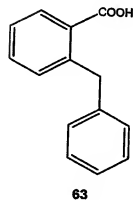
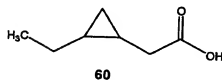
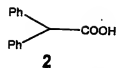
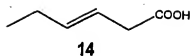
Position	conformationally rigid moieties
----------	---------------------------------

Ser₁Glu₄Lys₂₆Lys₂₇Asp₃₀Ser₁+Lys₂₇

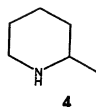
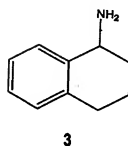
9. A peptide as claimed in claim 1 wherein said peptide sequence is GLP-1 and at least one conformationally rigid moiety is coupled with said GLP-1 peptide sequence via an amide bond at different positions as follows:

Position	conformationally rigid moieties
----------	---------------------------------

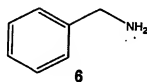
His₁



Glu₃

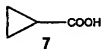


Asp₉

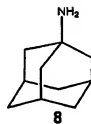


Position

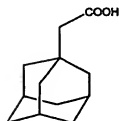
conformationally rigid moieties

His₁ + Glu₃

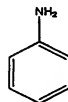
7



8

His₁ + Asp₉

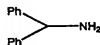
9



10

Glu₃ + Asp₉

11



12

10. A peptide as claimed in claim 1 wherein said peptide sequence is GLP-2 and at least one conformationally rigid moiety is coupled with said GLP-2 peptide sequence via an amide or ester bond at different positions of the peptide sequence.
11. A peptide as claimed in claim 1 wherein said peptide sequence is Enterostatin and at least one conformationally rigid moiety is coupled with said Enterostatin peptide sequence via an amide bond at different positions of the peptide sequence.
12. A peptide as claimed in claim 1 wherein said peptide sequence is NPY and at least one conformationally rigid moiety is coupled with said NPY peptide sequence via an amide or ester bond at different positions of the peptide sequence.
13. A peptide as claimed in claim 1 wherein said peptide sequence is NPYYP and at least one conformationally rigid moiety is coupled with said NPYYP peptide sequence via an amide or ester bond at different positions of the peptide sequence.
14. A peptide as claimed in claim 1 wherein said peptide sequence is Secretin and at least one conformationally rigid moiety is coupled with said Secretin peptide sequence via an amide or ester bond at different positions of the peptide sequence.
15. A peptide as claimed in claim 1 wherein said peptide sequence is Vasoactive Intestinal Peptide and at least one conformationally rigid moiety is coupled with said

Vasoactive Intestinal Peptide sequence via an amide or ester bond at different positions of the peptide sequence.

16. A peptide as claimed in claim 1 wherein said peptide sequence is Gastrin Inhibitory Peptide and at least one conformationally rigid moiety is coupled with said Gastrin Inhibitory Peptide sequence via an amide or ester bond at different positions of the peptide sequence.

17. A peptide as claimed in claim 1 wherein said peptide sequence is Vasostatin II and at least one conformationally rigid moiety is coupled with said Vasostatin II peptide sequence via an amide or ester bond at different positions of the peptide sequence.

18. A peptide as claimed in claim 1 wherein said peptide sequence is RANTES and at least one conformationally rigid moiety is coupled with said RANTES peptide sequence via an amide or ester bond at different positions of the peptide sequence.

19. A peptide as claimed in claim 1 wherein said peptide sequence is Eotaxin and at least one conformationally rigid moiety is coupled with said Eotaxin peptide sequence via an amide or ester bond at different positions of the peptide sequence.

20. A peptide as in any one of claims 1 to 18, wherein said conformationally rigid moiety is coupled with said peptide sequence via an amide or ester bond at the N-terminal.
21. A peptide according to any one of claims 8 to 19, wherein the conformationally rigid moiety has the formula 60 referenced in the description.
22. A peptide according to claim 20, wherein the peptide sequence is GLP-1.
23. Use of the peptide according to claim 22 in the treatment of glucose intolerance associated or not with insuline resistance pathologies.
24. Use according to claim 23 in the treatment of type II diabetes.
25. A peptide according to claim 1 wherein said peptide sequence is CGRP and at least one conformationally rigid moiety is coupled with said CGRP peptide sequence via an amide or ester bond at different positions of the peptide sequence.

(19) World Intellectual Property Organization
International Bureau(43) International Publication Date
7 February 2002 (07.02.2002)

PCT

(10) International Publication Number
WO 02/010195 A3(51) International Patent Classification: C07K 14/605,
14/00, 14/47, A61K 38/04, C07K 14/635, 14/655

(21) International Application Number: PCT/CA01/01119

(22) International Filing Date: 2 August 2001 (02.08.2001)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data: 60/222,619 2 August 2000 (02.08.2000) US

(71) Applicant (for all designated States except US): THER-
ATECHNOLOGIES INC. [CA/CA]; 2310, Alfred-Nobel
Boulevard, Saint-Laurent, Québec H4S 2A4 (CA).

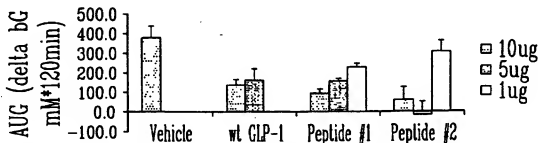
(72) Inventors; and

(75) Inventors/Applicants (for US only): GRAVEL, Denis
[CA/CA]; 207, des Pyrénées, St-Lambert, Québec J4S 1L3
(CA). HABI, Abdelkrim [CA/CA]; 7961, Champ d'Eau,
Anjou, Québec H1J 1X4 (CA). ABRIBAT, Thierry
[CA/CA]; 4659, Hutchison, Montréal, Québec H2V 4A2
(CA).(74) Agent: SWABEY OGILVY RENAULT; Suite 1600,
1981 McGill College Avenue, Montréal, Québec H3A 2Y3
(CA).(81) Designated States (national): AE, AG, AL, AM, AT, AU,
AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU,
CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH,
GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC,
LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW,
MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK,
SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA,
ZW.(84) Designated States (regional): ARIPO patent (GH, GM,
KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian
patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European
patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE,
IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF,
CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD,
TG).

Published:

— with international search report
— before the expiration of the time limit for amending the
claims and to be republished in the event of receipt of
amendments(88) Date of publication of the international search report:
3 October 2002For two-letter codes and other abbreviations, refer to the "Guid-
ance Notes on Codes and Abbreviations" appearing at the begin-
ning of each regular issue of the PCT Gazette.

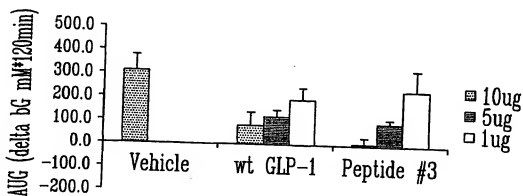
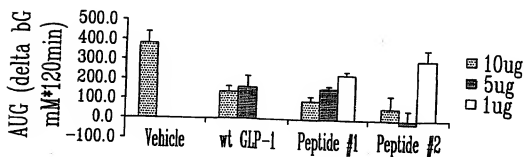
(54) Title: MODIFIED PEPTIDES WITH INCREASED POTENCY



(57) Abstract: The present invention is concerned with modified biological peptides providing increased potency, prolonged activity and/or increased half-life thereof. The modification is made via coupling through an amide bond with at least one conformationally rigid substituent, either at the N-terminal of the peptide, the C-terminal of the peptide, on a free amino or carboxyl group along the peptide chain, or at a plurality of these sites. Those peptides exhibit clinical usefulness for example in treating states of insulin resistance associated with pathologies such as type II diabetes.

WO 02/010195 A3

1/1

FIG - 1FIG - 2

SUBSTITUTE SHEET (RULE 26)

International Application No.

A. CLASSIFICATION OF SUBJECT MATTER					
IPC 7	C07K14/605	C07K14/00	C07K14/47	A61K38/04	C07K14/635
	C07K14/655				

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages
------------	--

Relevant to claim No.

A WO 00 14236 A (THERATECHNOLOGIES)
16 March 2000 (2000-03-16)
the whole document

7-9, 20,
21, 23, 24

A WO 96 37514 A (THERATECHNOLOGIES)
28 November 1996 (1996-11-28)
the whole document
& US 6 020 311 A (THERATECHNOLOGIES)
1 February 2000 (2000-02-01)
cited in the application

7-9, 20,
21, 23, 24

A	WO 98 08871 A (NOVO NORDISK) 5 March 1998 (1998-03-05) the whole document
---	---

9, 20, 21,
23, 24

-/-

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents:

A document defining the general state of the art which is not considered to be of particular relevance

E earlier document but published on or after the international filing date

L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another

*Or document referring to an oral disclosure, use, exhibition or

P document published prior to the international filing date but by other means

later than the priority date claimed

^T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled

5 document member of the same patent family

Date of the actual completion of the international search

16 July 2002

Date of mailing of the international search report

26/07/2002

Name and mailing address of the ISA
European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax (+31-70) 340-3018

Authorized officer _____

Masturzo, P

INTERNATIONAL SEARCH REPORT

International Application No

PCT/CA 01/01119

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 00 34332 A (SOCIETE DE CONSEILS, DE RECHERCHES ET D'APPLICATIONS SCIENTIFIQUES) 15 June 2000 (2000-06-15) the whole document	9,20,21, 23,24
A	WO 00 34331 A (SOCIETE DE CONSEILS ET D'APPLICATIONS SCIENTIFIQUES) 15 June 2000 (2000-06-15) the whole document	9,20,21, 23,24
A	WO 99 43707 A (NOVO NORDISK) 2 September 1999 (1999-09-02) the whole document	9,20,21, 23,24
A	US 5 093 233 A (M ROSENBLATT ET AL.) 3 March 1992 (1992-03-03) the whole document	8,20,21, 23,24
A	WO 98 01474 A (DOX-AL ITALIA) 15 January 1998 (1998-01-15) the whole document	7,20,21, 23,24
A	EP 0 187 622 A (SANDOZ-PATENT GMBH ET AL.) 16 July 1986 (1986-07-16) the whole document	7,20,21, 23,24
A	EP 0 030 920 A (CIBA-GEIGY) 24 June 1981 (1981-06-24) the whole document	7,20,21, 23,24

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 1-6, 10-25

Present claims 1-25 relate to an extremely large number of possible compounds and pertinent methods of use. Support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT is to be found, however, for only a very small proportion of the compounds and methods claimed. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Consequently, the search has been carried out for those parts of the claims which appear to be supported and disclosed, namely those parts relating to the compounds of claim 7-9 and pertinent methods.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA 01/01119

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Although claims 23-24 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☒ Claims Nos.: 1-6, 10-25
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/CA 01/01119

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 0014236 A	16-03-2000	US 6020311 A	01-02-2000
		AU 5500799 A	27-03-2000
		BR 9913515 A	05-06-2001
		WO 0014236 A2	16-03-2000
		DE 1109909 T1	23-05-2002
		EP 1109909 A2	27-06-2001
		ES 2164626 T1	01-03-2002
WO 9637514 A	28-11-1996	AT 204881 T	15-09-2001
		AU 697119 B2	24-09-1998
		AU 5683396 A	11-12-1996
		BR 9608799 A	07-12-1999
		CA 2222068 A1	28-11-1996
		WO 9637514 A1	28-11-1996
		DE 69614849 D1	04-10-2001
		DE 69614849 T2	16-05-2002
		DK 828758 T3	07-01-2002
		EP 0828758 A1	18-03-1998
		ES 2163020 T3	16-01-2002
		JP 11505807 T	25-05-1999
		PT 828758 T	28-02-2002
		US 6020311 A	01-02-2000
		US 5939386 A	17-08-1999
		US 5861379 A	19-01-1999
WO 9808871 A	05-03-1998	AU 732957 B2	03-05-2001
		AU 3847897 A	19-03-1998
		AU 4112497 A	19-03-1998
		BR 9711437 A	18-01-2000
		CN 1232470 A	20-10-1999
		CZ 9900629 A3	14-07-1999
		WO 9808871 A1	05-03-1998
		WO 9808872 A1	05-03-1998
		EP 0944648 A1	29-09-1999
		EP 0929576 A1	21-07-1999
		HU 9903714 A2	28-03-2000
		JP 2000500505 T	18-01-2000
		JP 3149958 B2	26-03-2001
		JP 2000517308 T	26-12-2000
		JP 2001011095 A	16-01-2001
		NO 990950 A	28-04-1999
		PL 331896 A1	16-08-1999
		US 6268343 B1	31-07-2001
		US 2001011071 A1	02-08-2001
		US 2002025933 A1	28-02-2002
WO 0034332 A	15-06-2000	AU 1751200 A	26-06-2000
		BR 9916027 A	28-08-2001
		CN 1342166 T	27-03-2002
		CZ 20011895 A3	12-12-2001
		EP 1137666 A1	04-10-2001
		NO 20012787 A	06-08-2001
		WO 0034332 A1	15-06-2000
WO 0034331 A	15-06-2000	AU 1973600 A	26-06-2000
		BR 9915961 A	21-08-2001
		CN 1329620 T	02-01-2002
		CZ 20011748 A3	13-02-2002

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/CA 01/01119

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 0034331 A		WO 0034331 A2 EP 1137667 A2 NO 20012786 A	15-06-2000 04-10-2001 20-07-2001
WO 9943707 A	02-09-1999	AU 2610699 A AU 2610799 A AU 2610899 A WO 9943706 A1 WO 9943341 A1 WO 9943707 A1 EP 1060191 A1 EP 1061946 A1 EP 1062240 A1 JP 2002504518 T JP 2002512175 T JP 2002506792 T US 6268343 B1 US 2001011071 A1 ZA 9901569 A ZA 9901570 A	15-09-1999 15-09-1999 15-09-1999 02-09-1999 02-09-1999 02-09-1999 20-12-2000 27-12-2000 27-12-2000 12-02-2002 23-04-2002 05-03-2002 31-07-2001 02-08-2001 27-08-1999 02-09-1999
US 5093233 A	03-03-1992	NONE	
WO 9801474 A	15-01-1998	IT MI961408 A1 AU 3693097 A WO 9801474 A2 EP 0914342 A2	08-01-1998 02-02-1998 15-01-1998 12-05-1999
EP 187622 A	16-07-1986	DE 3511206 A1 AU 5184886 A DK 4686 A EP 0187622 A2 ES 550692 D0 ES 8705469 A1 FI 860029 A GR 860010 A1 HU 42101 A2 JP 61161300 A US 4728638 A ZA 8600115 A	09-10-1986 10-07-1986 08-07-1986 16-07-1986 01-05-1987 16-07-1987 08-07-1986 30-04-1986 29-06-1987 21-07-1986 01-03-1988 26-08-1987
EP 30920 A	24-06-1981	AT 6251 T AU 544256 B2 AU 6534580 A CA 1161033 A1 DD 154098 A5 DE 3066654 D1 DK 532180 A EP 0030920 A2 ES 497696 D0 ES 8200861 A1 FI 803849 A GR 73621 A1 JP 56092850 A KR 8500415 B1 KR 8500414 B2 NO 803757 A NZ 195817 A	15-03-1984 23-05-1985 18-06-1981 24-01-1984 24-02-1982 22-03-1984 15-06-1981 24-06-1981 16-11-1981 16-02-1982 15-06-1981 26-03-1984 27-07-1981 26-03-1985 26-03-1985 15-06-1981 06-07-1984

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/CA 01/01119

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 30920	A	PT 72191 A , B	01-01-1981
		US 4369179 A	18-01-1983
		ZA 8007796 A	27-01-1982

THIS PAGE BLANK (USPTO)